DO "CARRIER" STRAINS DIFFER FROM STRAINS ISOLATED FROM ORDINARY TYPHOID CASES ?

EXPERIMENTAL TYPHOID-PARATYPHOID CARRIERS. III

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This question involves the consideration of several factors which may play a part in the successful production of a high percentage of experimental gallbladder or renal carriers. It should be emphasized that this study was primarily undertaken to elucidate certain facts recorded in the literature pertaining to animal carriers. It was not the intention to attempt to prove or to refute the conception that the typhoid strains isolated from human carriers may differ in their invasive or infective properties, a phase of the problem which is practically impossible when one considers that the experimental reproduction of typhoid fever in laboratory animals cannot be accomplished. The customary method of testing the virulence of typhoid strains by intraperitoneal injections into guinea-pigs does not reproduce the conditions as they operate in human infections and the data collected by this method cannot be used for comparison. Neither Lentz [1] nor Ledingham [2] noted by the use of this procedure differences between the virulence of the carrier strains and those isolated from ordinary typhoid cases. Some incomplete observations of Ledingham, however, suggest that the virulence of the typhoid strains isolated from one and the same carrier may vary for guinea-pigs at different times of the year. For example, a strain isolated from an intestinal carrier exhibited a reduced virulence during the winter months. Using a similar method of testing for virulence, Remlinger [3] noted that typhoid bacilli found in liver abscesses were avirulent, and Niepratschk [4] isolated from a renal carrier a strain of low virulence. Furthermore, Levy and Gaehtgens [5] recorded by means of bacteriotropin tests the observations

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* Dr. C. R. Christiansen, who died from influenza in October, 1917, conducted during the years 1915-16 the majority of the experiments to be reported. This paper should stand as a memorial to his enthusiastic and resourceful collaboration in our typhoid studies.

1 Klin. Jahrb., 1905, 14, p. 475.

2 The Carrier Problem in Infectious Diseases, London, 1912, p. 110.

3 Compt. rend. Soc. de biol., 1897, 4, p. 110.

4 Ztschr. f. Hyg. u. Infektionskr., 1909, 64, p. 454.

5 Ergebn. d. allg. Path. u. path. Anat., 1915, 18, p. 491.

that the virulence of the typhoid bacilli excreted by typhoid patients was as a rule greater than the one of carrier strains. It. has been suggested by Hilgermann [6] that the relative infrequency of infections in the entourage of carriers may be due to variations in the virulence of the organism and that this diminishes with the continued parasitic existence of the latter in the organs of its host. Based on some observations on rabbits Wagner and Emmerich [7] came to the same conclusions. The well-known studies by Metchnikoff and Besredka [8] on experimental typhoid fever demonstrated that typhoid bacilli shed by a chronic carrier are pathogenic for small laboratory animals, but that they are unable to transmit typhoid to chimpanzees. The general notion that carrier strains are less virulent than those isolated from acute typhoid cases finds, therefore, some justification, and the question of the occurrence of saprophytic and parasitic strains is again placed in the foreground. Closely interwoven with the answer to the original question is the consideration of the possibility that carrier strains may possess specific elective properties for the tissues from which they are isolated. Ledingham,[9] in discussing this subject, expresses the opinion that this hypothesis can be proved. A carrier strain that has sojourned for long periods in man may conceivably be more highly endowed with those properties which permit of its continued vegetation within the body after the infection is past than a typhoid strain responsible for a water-borne epidemic, which gets little opportunity of becoming accommodated to the human organism. As far as we know, the conception of elective organotropism in the sense of Rosenow [10] has not been considered experimentally for the typhoid bacillus but has been fully proved by Fränkel and Much [11] for a paratyphoid B bacillus obtained from a case of purulent cholecytitis.

Early in the course of our typhoid work, we felt the necessity of studying these problems pertaining to the inherent properties of the typhoid bacillus before we undertook a systematic study of the factors responsible for the occurrence of gallbladder, liver or renal carriers. We were fortunate in having access to carrier strains, which were responsible for extensive and serious epidemics. The dynamic properties of some of these strains as far as they concern man had been well

[6] Klin. Jahrb., 1908, 19, p. 463.
[7] Med. Klin., 1916, 12, p. 819.
[8] Ann. d. l'Inst. Pasteur, 1911, 25, p. 193.
[9] The Carrier Problem in Infectious Diseases, pp. 75 and 76.
[10] Jour. Infect. Dis., 1916, 14, p. 527.
[11] Ztschr. f. Hyg. u. Infektionskrankh., 1911, 69, p. 342.

investigated epidemiologically. These investigations were at our disposal through the publications and verbal informations obtained from Drs. W. A. Sawyer and F. G. Cummings. Additional cultures were isolated from carrier cases in the wards of the University of California Hospital and compared with recently isolated strains of our own and those we received through the courtesy of Drs. H. T. Chickering, J. V. Cooke, and the Bureau of Communicable Diseases of the California State Board of Health. The available carrier strains were not very numerous, but could at least be considered representative of the variety of typhoid bacilli as they occurred in a large community.

The analysis of these typhoid strains covered originally two questions: First, are there any cultural or biochemical differences between carrier strains and those recently isolated from acute typhoid cases? Second, do carrier strains exhibit a specific elective behavior for certain tissues in the rabbit, and can this elective localization be acquired by successive passage through the gallbladder of this species of animal? A study of carrier strains isolated at different time intervals from one and the same case was contemplated, but the specimens necessary for this investigation could not be procured with desirable regularity.

In the course of the analysis of the preliminary data, it was realized that an additional question could be advantageously investigated on the same animals, namely, Do immunized rabbits develop a gallbladder carrier state more readily than normal animals? This question was first raised by Fornet [12] in connection with his theory that the carrier state is to be regarded as a consequence of established partial immunity to the typhoid bacillus, and that, in fact, the bacilli discharged by the carrier may not necessarily be descendants of those that caused the primary infection. A reinfection takes place, which, according to his views, is not accompanied by any clinical symptoms. The region attacked by the bacilli in this reinvasion would have acquired, in consequence of the primary infection, such a tolerance to the micro-organisms as to render its saprophytic existence possible. Fornet mentioned, further, in his first article that animal experiments to support his contention were in progress. According to a more recent review,[13] he apparently gained from a few preliminary experiments the impression that immunized rabbits developed more readily and more regularly a typhoid carrier state by alimentary infection than nonimmunized

[12] Ztschr. f. Hyg. u. Infektionskrankh., 1909, 64, p. 365.
[13] Ergebn. d. innern Med. u. Kinderheilk., 1913, 11, p. 205.

animals. In the same article,[14] he suggested that similar results may be obtained in animals infected by the intravenous route. We will have occasion to discuss, in a paper dealing with the feeding of typhoid and paratyphoid bacilli to rabbits, the impossibility of producing a gallbladder infection by the alimentary method. This phase of Fornet's theory is unquestionably incorrect. One may recall in this connection that the carrier state supposed to have been produced by the inoculation of one half of a standard 10% blood-agar culture of a certain strain of B. typhosus, has been recommended as a means for testing the immunizing power of a given typhoid vaccine. Nichols [15] and others were unable to repeat these results. The experiments of the latter are supported by our own observations, namely, that typhoid immunized rabbits develop a carrier state in a higher percentage of instances than normal animals. The occurrence of local infections in immunized or possibly sensitized animals has some bearing on the general problem of focal infections in man. Numerous observations reported in the literature indicate that immunization does not prevent the development of local infections in rabbits. One gains the impression that such procedures quite frequently favor it. In this connection the work of Faber [16] on streptococcic arthritis, and of Wadsworth [17] on pneumonia in rabbits, should be mentioned. Furthermore, Creig [18] reports the development of a gallbladder carrier state in rabbits progressively immunized with living cholera-like vibrios, while Flu [19] notes similar lesions in animals repeatedly infected with Flexner dysentery bacilli. The factors responsible for these results have been investigated, and will be discussed in this and in subsequent papers.

THE CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF "CARRIER" STRAINS

During the last five years 14 B. typhosus strains, isolated from carriers in California, were studied in detail by means of the usual cultural and carbohydrate tests. The histories of the strains used in the animal experiments are indicated in table 1. The epidemics caused by strains 10 and 21, are discussed by Sawyer [20] and the infections

[14] Ibid., p. 216.
[15] Jour. Exper. Med., 1914, 20, p. 573.
[16] Jour. Exper. Med., 1915, 22, p. 615.
[17] Am. Jour. Med. Sc., 1904, 128, p. 851.
[18] Indian Jour. Med. Research, 1915-16, 3, pp. 259 and 397.
[19] Geneesk. Tidjschr. v. Nederl. Indie, 1918, 58, p. 67.
[20] Jour. Am. Med. Assn., 1912, 58, p. 1336; 1914, 63, p. 1537.

provoked by strain 4 have been reported by Cummings.[21] Strains 18, 49, 1 and 84 are stock strains, or were isolated by us from necropsy material, or from secretions of carriers under our control. These cultures, in addition to 7 others, were studied by the methods recently described, in connection with the analysis of 2 irregular typhoid strains.[22] No differences from the ordinary typhoid strains could be noted; the behavior on carbohydrate medium, milk and on rhamnose plates was typical. All the strains were rapid xylose fermenters and failed to attack dulcite in liquid mediums. These observations are quite in harmony with the findings of E. Müller,[23] who studied 19 carrier strains. According to his results, the strains of B. typhosus obtained from intestinal carriers showed no abnormalities, nor did the cultures exhibit any indication of newly acquired properties.

All strains were specifically agglutinated by a polyvalent typhoid immune serum. The variation of the individual strains differed in no respect from those commonly encountered by Bull and Pritchett [24] with the ordinary typhoid strains. As most of our tests were made previous to the findings of Hooker,[25] who clearly demonstrated consistent antigenic differences among some strains of B. typhosus, absorption tests were not done with the freshly isolated strains, and our inquiry along these lines is therefore incomplete. Recently a few tests conducted with the strains cultivated on agar for from 2-7 years showed that strains 10, 4 and 84 belonged to group 2 and strains 18 to group 3 of Hooker's classification, while strains 21, 49 and 1 exhibit the characteristics of group 1. Hooker had already found, in his studies in 1916, that our strain 18 and his strain 4 fell in group 3. It is not unlikely that such tests would have produced different results had they been carried out when the strains were originally isolated. The existence of atypical and irregular typhoid strains in carriers should be seriously considered in the light of our recent observations. Moreover, the publication of Downs [26] suggests the presence of antigenic differences among typhoid strains and a subdivision into at least 5 groups. Confirmation of these observations as to the occurrence of various types of typhoid bacilli would be of considerable epidemiologic importance in the analysis of "carrier" borne typhoid outbreaks, and may perhaps

[21] Jour. Am. Med. Assn., 1917, 68, p. 1163.
[22] Meyer, K. F., and Neilson, N. M.: Jour. Infect. Dis., 1920, 27, p. 46.
[23] Centralbl. f. Bakteriol. I. 1909, 53, p. 209.
[24] Jour. Exper. Med., 1916, 24, p. 35.
[25] Jour. Immunol., 1917, 2, p. 1.
[26] Abstr. Bacteriol., 1920, 4, No. 56, p. 19.

explain the variable infectivity of carriers. Gruber,[27] for example, was able to trace a carrier through the peculiar behavior in glycerol of the causative typhoid strain, the so-called B. metatyphosus of Mandelbaum. An intensive study of carrier strains would perhaps also explain the findings of Schlemmer,[28] who noted that one and the same carrier may eliminate at varying time intervals strains of typhoid bacilli, which are nonsusceptible to the bactericidal properties of the same immune serums. These observations involuntarily lead to a consideration of the virulence of our carrier strains. There is no experimental animal on which one can successfully test the relative virulence of typhoid strains, but to satisfy our curiosity we applied the method of Ledingham [29] to strains 1, 49 and 84. These organisms killed guinea-pigs weighing 250 gm. in quantities of not less than 1 c c of a 24-hour old broth culture. Strain 49 killed in 2.5 c c amounts. In comparison with the ordinary typhoid strains 40, 41, 54, 55 and 56, which were fatal to guinea-pigs in 1-2 c c amounts, the virulence of the 3 carrier strains cannot be considered exceptionally low.

Our findings on carrier strains differ therefore in one respect from those reported by Lentz, Ledingham, E. Müller and others, and justify the definite and final conclusion that culturally and biochemically carrier strains do not differ in any way from those recently isolated.

THE BEHAVIOR IN THE RABBIT OF CARRIER STRAINS AND OF STRAINS RECENTLY ISOLATED FROM ACUTE INFECTIONS

In accordance with the problems discussed in the introduction, we tested the theory of Fornet and investigated the conception of elective organotropism advanced by Rosenow and Brown,[30] using a number of carrier strains at our disposal. These tests were paralleled by similar experiments with recently isolated strains and with those artificially cultivated for several years. Such a comparison was necessary in order to prove the following points: (a) the validity of the conclusions drawn from the experiments conducted with carrier strains; (b) the observations of Weinfurter,[31] Nichols,[15] and others which indicated that freshly isolated strains produced gallbladder lesions in a higher

[27] Arch. f. Hyg., 1913, 80, p. 272.
[28] Ztschr. f. Immunitätsforsch. u. exper. Therapie, 1911, 9, p. 149.
[29] The Carrier Problem in Infectious Diseases, pp. 109 and 110.
[30] Arch. Int. Med., 1919, 23, p. 185.
[31] Centralbl. f. Bakteriol., I. O., 1915, 75, p. 379.

percentage of instances than old strains; and (c) the assumption that a "certain" strain of B. typhosus cultivated on blood agar is necessary to provoke a carrier state in rabbits. Furthermore, our ulterior motive in conducting these tests centered on the desire to find a typhoid strain which was particularly suited for carrier studies on rabbits.

Methods.—The rabbits used in this series of experiments weighed between 2,000 and 3,000 gm., and were supplied from the same source. During the years 1915, 1916, and 1917 these animals were free from coccidiosis and spontaneous cholecystitis. Little attention has been paid to this infection by the majority of workers on experimental typhoid, and sweeping conclusions on gallbladder infections have been drawn from the results obtained on rabbits, which possessed, according to the published protocols, livers heavily infested with coccidiosis. Some investigators (Gotschlich[32]) are aware of the fact that coccidiosis of the liver favors the localization of bacteria in the biliary passages and the gallbladder, while others intentionally or unintentionally ignore this infection. Experimental conclusions on cholecystitis appear justified only when one is certain that the animal does not possess predisposing infections. Many statements relative to the value of a method for the production of carriers, expressed in percentages of infections, are seriously invalidated by the utter neglect of these fundamental requirements.

The animals were kept on a mixed oat, hay and cabbage diet, in single "Lewis" cages and in a fly proof isolation room. The sawdust bedding was disinfected by lysol or strong lye solutions.

Immunization was accomplished by at least 6 intravenous inoculations, at 6-7 day intervals, of heat killed (53-54 C.) (McCoy), tricresolized, autogenous cultures grown on rabbit-blood agar. The vaccine amounts were increased progressively from 1/20 to 1/2 of a slant. Ten to 15 days after the last injection of the vaccine, the animals were infected with the same strain of typhoid bacilli in a living state. For these tests the cultures were purified on Endo plates, and kept on veal agar or grown on rabbit-blood agar slants for at least 5 generations before use. The preparation of the mediums, the selection of the tubes and proper slanting were conducted in accordance with the specifications ordinarily employed. We intend to discuss in another paper of this series the observations made in connection with the cultivation of typhoid strains on rabbit-blood agar, but attention is now called to the fact that in our experience never more than 40-60,000 million typhoid bacilli grew on the specified agar surface. The figures determined by Gay and Claypole and faithfully copied by Stone,[33] namely, 1,400,000 million organisms, are probably incorrect. In order to reduce the initial mortality caused by the inoculation of large doses of living typhoid bacilli into rabbits, we determined when possible the minimum lethal dose, and found that from 2-5 billions per kilogram of rabbit weight are fairly well borne by normal animals weighing over 2,000 gm. Expressed in terms pertaining to blood-agar slants, these amounts varied from 1/10 to 1/4 slant per rabbit. All inoculations were made with unstrained suspensions in sterile saline solution using a marginal vein, and in one series of animals the renal artery.

[32] Handb. d. path. Mikroorganism, Ed. 2, 1912, 1, p. 217.

[33] Jour. Infect. Dis., 1919, 25, p. 290.

The rabbits were killed in from 20-35 days after the inoculation. Cultures were made on Endo-agar plates or by enrichment of portions of the organs in 10% ox bile rabbit broth. The major portion of the data presented was collected early in the course of our study on experimental typhoid carriers. The conception of the carrier state at this period was comparatively simple, namely, the presence of typhoid bacilli in the bile and gallbladder wall constituted a positive result.

The data presented in tables 1 and 2 should be considered from this point of view. An animal was noted as "died" when it succumbed to the intoxication in the first 24-48 hours.

TABLE 1

GALLBLADDER CARRIERS PRODUCED IN RABBITS BY THE INJECTION OF OLD AND RECENT "CARRIER" STRAINS OF B.TYPHOSUS

Strain	Age of Strain	No. of Animals		Positive		Negative	
		Immune	Normal	Immune	Normal	Immune	Normal
Stool and Osteomyelitis:							
10 "O" responsible for 26 contact cases in 3½ years	3½ years	5	5	1	1	4	4 (died)
18 "Cr" tibial abscess 2 years after an attack of typhoid fever	3 years	4	2	1	0	3	2
21 "L" responsible for 93 cases in one epidemic	1 month	6	8	5	5	1	3 (died)
4 "H" responsible for 23 cases in a food epidemic	2 months	3	—	3	—	—	—
49 gallstone, negative, no history of typhoid fever	3 days	3	3	0	1	3	2
Urine:							
"1" renal carrier, injection made through the left renal artery	3-4 months	6	2	6	2	0	0*
84 "renal carrier," right typhoid pyelonephritis	Immediately urine sediment of 200 c c of urine 10 billion organisms	2	2	1	2	1	0*
7 strains................	Immediately to 3½ years	29	22	17 56%	11 50%	12 44%	19 50% 9% died

* Kidneys of all animals negative.

Experimental Data.—The various experiments are shown in concrete form in tables 1 and 2. It is quite apparent that with the exception of strains 21 and 1, none of the carrier strains possessed elective properties for the gallbladder of rabbits. The percentage of infections in 51 rabbits inoculated with carrier strains was lower than that found in 70 rabbits similarly infected with recently isolated strains of B. typhosus. The highest percentage of infections was noted with a freshly isolated strain 21 which was introduced intravenously, and with a 3-4 month old culture (1) which was injected through the left renal artery. The latter experiment will be discussed elsewhere more in detail, but in connection with it attention should be called to the fact

that operative technic, fasting of the animals with the resulting stasis in the gallbladder, unquestionably contributed to the permanency of the infection in the biliary passages. Furthermore, this experiment with strain 1, as well as that conducted with 84, lend little support to the contention that the parasitic typhoid strains isolated from chronic renal carriers possess for the rabbit at least elective organotropic properties.

TABLE 2

GALLBLADDER OR LIVER CARRIERS PRODUCED IN RABBITS BY THE INJECTION OF RECENT AND OLD STRAINS OF B. TYPHOSUS ISOLATED FROM STOOL BLOOD CULTURES

Strain	Age of Strain	No. of Animals		Positive		Negative	
		Immune	Normal	Immune	Normal	Immune	Normal
Recent Blood Cultures:							
24	28 days	10	12	8	5	2	7
26	55 days	2	2	1	2 (died)	1	0
27	6 months	1	1	1	1	0	0
28	5 months	1	2	0	0	1	2
29	5 months	2	2	1	1 (died)	1	1
36	53 days	1	2	0	1	1	1 (died)
37	2 months	1	1	1	0	0	1
38	1½ months	1	1	0	0	1	1
40	2½ months	1	1	0	1	1	0
41 (severe case)	3 months	1	1	1	1 (died)	0	0
45	3½ months	1	2	0	1 (died)	1	1
46	2 months	1	1	1	0	0	1 (died)
48	1 month	2	1	2	1	0	0
50	1 month	1	1	1	1	0	0
52*	2½ months	2	2	0	1	2	1 (died)
53*	2 months	1	1	1	1 (died)	0	0
54*	2 months	1	1	1	1	0	0
55*	1 month	1	1	1	1	0	0
56*	1 month	1	1	1	0	0	1
Chr. 2	4 months	1	1	1	1	0	0
Total		33	37	22	20	11	17
Average percentage of carriers				66%	54.3% (25% died)	33%	45% (17% died)
Old Stock Cultures:							
15 ("I")	3 years	2	0	1	0	1	0
17 (old)	>3½ years	1	—	0	—	1	—
14 (Hopkins)	>4 years	2	2	0	0	2	2
6 (Rawlings)	15 years	9	5	2	0	7	5
23 (Dorset)	16 years	2	2	1	1	1	1
Total		16	9	4	1	12	8
Average percentage of carriers				25%	11%	75%	88.8%

Quite recently Lentz, Hailer and Wolf [34] reported similar tests with eight carrier strains. Each culture was inoculated in one loopful amounts into 5 rabbits. Only 30% of the animals developed a carrier

state. These tests were undertaken to prove whether or not the strains employed by Doerr [35] and by Johnston [36] possessed specific elective properties, which could explain the high percentage of infected gallbladders recorded by these writers. It is evident that our experiments confirming those of Lentz [34] fail to support the conception of elective parasitism.

Our observations support the findings of Weinfurter [31] and of Nichols,[15] namely, that recently isolated parasitic strains produce a higher percentage of carriers than old saprophytic strains. But it is also demonstrated that even such strains failed to produce biliary infections regularly. The high initiated mortality of 17-25% among the nonimmunized rabbits must, however, be considered a serious and expensive disadvantage.

How do our results compare with those reported by 15 other workers? We have attempted to analyze their reports and have summarized the data in table 3. In this summary it is difficult to estimate the number of organisms inoculated, the weight and age of the rabbits, and the time period elapsing between infection and necropsy, facts which interfere seriously in estimating the true value of the experiments. Making allowance for these and other factors like coccidiosis, individual resistance, diet, etc., it is quite obvious that the average percentage of gallbladder carriers produced does not exceed 65%. This figure may be accepted for our analysis. A variety of strains was employed by the different experimenters. Old and recently isolated strains and even carrier strains were chosen by Morgan,[37] by Hailer and Rimpau,[38] by Hailer and Ungermann,[39] and by Lentz, Hailer and Wolf.[34] The evidence supports our conclusions that recently isolated strains, whether of carrier or acute typhoidal origin, produce gallbladder infections more frequently than old stock cultures. Carrier strains offer no advantage over recently isolated strains of acute typhoid cases. In our opinion the recognition of the fact that a large number of organisms is necessary to insure biliary infection is important. In this connection the high percentage of deaths should also be considered. Most of the workers in this field of experimental pathology fail to state the actual loss of animals in the 24 to 72 hours

[35] Centralbl. f. Bakteriol. I. O, 1905, 39, p. 624.
[36] Jour. Med. Research, 1917, 37, p. 189.
[37] Jour. Hygiene, 1911, 11, p. 202.
[38] Deutsch. med. Wchnschr., 1912, 38, p. 2267.
[39] Arb. a. d.Gsndhsamte, 1914, 47, p. 451.

following the injection of large doses of living typhoid bacilli. In our series we recorded as high as 25% mortality in nonimmunized animals, and we intend to publish several series of tests in which the mortality rose to 50%. Every worker will agree that such a method is prohibitive and wasteful. It will be the purpose of subsequent papers to discuss the procedures that were found of value in overcoming this loss. By these methods it was possible to raise the percentage of positive carriers to from 90-100% of the animals infected.

TABLE 3

PERCENTAGE OF GALLBLADDER CARRIERS PRODUCED BY VARIOUS WORKERS

Author	Weight of Rabbits	Number of Bacteria Injected		Percentage of Carriers
		As Stated by Author	Estimated by Us	
Forster and Kayser	2.0 - 2.6	0.5–0.6 mg.	600–15,000 million	Not regular infected on 5th day
Doerr	1.13- 2.0	¼–2 loopful	600- 5,000 million	90% in 10 rabbits
Lemiérre and Abrami	—	2 c c of Widal's agglutination fluid	1,000- 2,000 million?	Irregular after 6th day, recovery
Chiarolanza	2.0 - 2.5	½–4 loopful	1,250–10,000 million	74% in 23 rabbits
Blumenthal, E.	—	1 loopful	2,500 million per kg. = 5-9,000 million per animal	100% in 5 rabbits
Bully	1.16- 2.0	1 loopful per kg.	2,500 million per kg. 2,500-5,000 per animal	48% in 40 contral rabbits
Morgan	Aver. 2.52	4 c c of broth	2,800–3,200 million	50% in 10 rabbits
Johnston	0.98-1.94	0.5 c c of an agar suspension	?	?
Perussia	—	1-4 loopful per kg.	2,500–10,000 million per kg.	46% carriers in 15 rabbits
Weinfurter	2.5	¼-3 loopful	600–7,500 million	53% in 58 rabbits
Gay and Claypole	2.0-2.5	½ standard blood agar slant 720,000 million (?)	20,000–25,000 million	93.0% in 28 rabbits 90.6% in 43 rabbits 74-76% in ? rabbits (Beckwith)
Cummins and Cumming	—	1/10 slant	2,500–4,000 million	71% in 7 normal and immune rabbits had liver infected; 29% in 7 rabbits had infected gallbladder
Nichols	2.0-3.0	½ fatal dose = 1/10 slant of blood agar	4-5,000 million	21% in 16 rabbits, 28.8% in 45 rabbits, 40% in 10 immune rabbits
Hailer and Rimpau and Hailer and Ungermann	2.5-3.0	1 loopful per kg.	6,000–7,000 million per animal	75% in 61 rabbits killed 8-34 after injection
Lentz, Hailer and Wolf	—	1 loopful	2-5,000 million	30% in 40 rabbits
Teague and McWilliams	1.4-2.4	Large dose 8,000–15,000 million	—	60% positive in 10 rabbits 3-24 days
Stone	1.7-4.3	⅙ blood-agar slant 460,000 million (?)	10–20,000 billion	92% (Beckwith states 94%) in ?
Beckwith	—	⅙ blood-agar slant	10–20,000 billion	100% in ? rabbits

Analyzing the data in tables 1 and 2, we found to our surprise that immunized rabbits became carriers more readily than nonimmunized animals, an observation which confirmed the theory of Fornet, to which

we had had no access at the time our deductions were originally drawn. Nichols [15] also noted a higher percentage of infection among his immunized rabbits, as did Cummins and Cumming.[40] Certain requirements must be fulfilled to obtain these results. It was found that immunized rabbits eliminated a larger number of typhoid bacilli than normal animals when the test inoculation was made on the 6-12th day after the last injection of the vaccine. Additional factors unquestionably play an important rôle, but it is not the purpose of this paper to discuss them. So much can, however, be said: The evidence at our disposal does not permit us to conclude that the results obtained on immunized rabbits are applicable to man and to the human carrier state.

Furthermore, it is evident that the development of the carrier state in rabbits cannot be used as a test for the potency of vaccines. Our rabbits were protected by 6 to 7 inoculations of a fresh vaccine prepared from the strain which was used for the test inoculations. Moreover, the intravenous doses were not larger than those usually employed. It has been argued that Nichols obtained his gallbladder infections in immunized rabbits because the intravenous test dose of one half fatal dose = 1/10 slant = 4-5 billion organisms was sufficient to overcome the increased bactericidal effect of the blood (which, by the way, does not exist). If this argument is sound, the same conclusion must apply to those studies, in which about 720 billion (?) living B. typhosus were employed and in which the reverse is reported as having occurred. In this connection one may state that even immunization with living bacteria does not alter the final outcome. Independent of the degree of protection, a certain percentage of rabbits that have recovered will develop the carrier state. This result is not surprising when one appreciates that, according to Parker and Franke,[41] whose studies we have confirmed by extensive experimentation, the destruction of intravenously inoculated typhoid bacilli progresses with the same speed in immunized as in normal rabbits. No difference can be recorded in the bactericidal power of normal and immunized rabbit tissues. The infection of the gallbladder and the persistence in it of viable bacteria depend on certain factors operative in the liver, its vascular system, the bile and the diet of the animal (coccidiosis excluded) and not on the bactericidal properties of the blood.

[40] Jour. Roy. Army Med. Corps, Lond., 1914, 22, p. 378.
[41] Jour. Med. Research, 1919, 39, p. 301.

CAN TYPHOID STRAINS ACQUIRE ELECTIVE CHOLECYSTOTROPIC PROPERTIES BY SUCCESSIVE PASSAGE THROUGH THE GALLBLADDER ?

The studies reported in this chapter were undertaken in 1915-16, when the influence of Rosenow's doctrines permeated the bacteriologic and medical literature. Elective localization had been induced with the plague bacillus, the staphylococcus, the streptococcus, the colon bacillus, the paratyphoid bacillus and perhaps the representatives of the Brucella group, but no reports on the typhoid bacillus were available. As we were searching in our carrier studies for an organism which regularly localized in the gallbladder, the contemplation and execution of a series of passage experiments had at that time some justification and interest. Today, after having analyzed the mechanism of this infection in the rabbit, our efforts of this period appear superfluous. The course of the experiment is shown in tabulated form in table 4. By direct inoculation into the gallbladder the recently isolated typhoid strain was perpetuated through 4 rabbits. Each successive infection was done by transference of the infected bile from the viscus of the preceding, laparotomized rabbit. The strain was lost in the third passage; 15 days after the inoculation into Rabbit 722 the gallbladder bile was found sterile. This observation proves in the first place the contention of Hailer and Ungermann [39] that even the method of intravesicular inoculation of typhoid bacilli is not absolutely dependable, and that persistence of the bacteria may be of comparatively short duration. Furthermore, this typhoid strain, after it had been resident in the gallbladder for 116 days, had not acquired any selective property for growth in this viscus. This conclusion is also supported by the tests conducted on 33 rabbits, which received intravenously the first or second generation of the bile culture on blood-agar slants. The percentage of carriers (45 and 22%) differed in no respect from that noted for the recently isolated, nonlapinized strains. One may, therefore, conclude that elective cholecystotropic properties cannot be conferred on typhoid strains by successive passage through rabbits. This conclusion has, during the past 4 years, been repeatedly confirmed by experiments with typhoid or paratyphoid strains, which had been resident in the gallbladder of the rabbit from 230 to 816 days. In not one instance did these carrier strains exhibit elective properties. Prolonged sojourn in the rabbit tissues produced changes in agglutinability, as has been observed by Lange and Roos [42] and by Wagner

[42] Arb. a. d. Kais. Gsndhtsamte, 1915, 50, p. 57.

and Emmerich.[7] Nearly 50% of the gallbladder strains were temporarily inagglutinable. A paratyphoid A strain, which persisted for 816 days in the gallbladder and the liver, was hyperagglutinable and clumped spontaneously. Intravenous injections of 10,000 million bacteria of this strain (second generation) were well tolerated by 5 rabbits. At necropsy 10 to 25 days after the injection these animals exhibited normal gallbladders. The strain had undergone some fundamental changes, but had not acquired specific invasive properties. An explanation of the carrier state can therefore not be expected on the basis of an elective localization in the sense of Rosenow.

TABLE 4

Passage of a Recently Isolated Strain of B. Typhosus (K) Through the Gallbladder of Several Rabbits

1st Passage.	Rabbit 790 (2d generation intravesicularly) 14 days	Rabbit 723 intravenously 1st generation, positive Rabbit 724 intravenously 1st generation, positive Rabbit 725 intravenously 1st generation, negative Rabbit 726 intravenously 1st generation, negative Rabbit 791 intravenously 2d generation, positive (died) Rabbit 792 intravenously 2d generation, positive Rabbit 793 intravenously 2d generation, negative (died) Rabbit 794 intravenously 2d generation, negative Rabbit 795 intravenously 2d generation, negative (died) Rabbit 796 intravenously 2d generation, negative Rabbit 798 intravenously 2d generation, positive
2d Passage.	Rabbit 797 (bile of 790 intravesicularly) 35 and 70 days, resp.	5th generation of 790 and 2d generation of 797 8 immune rabbits, all negative 14 normal rabbits, 5 died in 24 hours, positive 2 positive on necropsy 7 negative on necropsy
3d Passage.	Rabbit 722 (bile of 797 intravesicularly) Negative on 25th day	

SUMMARY

A comparative study of 14 carrier strains failed to reveal any striking differences between these strains and those isolated from acute typhoid cases. Seven carrier strains tested on rabbits by means of intravenous injections failed to exhibit specific elective cholecysto- and renotropic properties. Furthermore, it was impossible to confer such characteristics to a recently isolated strain of B. typhosus by successive passage through the gallbladder of rabbits.

It was, however, demonstrated that immunized rabbits inoculated with large doses of living typhoid bacilli exhibited gallbladder infections in a somewhat higher percentage of instances than normal rabbits. The theory of Fornet and the publications of other workers on experimental typhoid carriers are discussed and compared with our own observations.